REMARKS

To expedite prosecution of preferred embodiments, Applicants have amended the claims as follows. Claim 1 has been amended to use of primer extension reaction and detection method of MALDI-TOF mass spectrometry. Support for the amendments can be found throughout the specification and claims 5 and 8, as originally filed. Claims 12 and 13 have been amended to refer to "at least" 25 or 50 target nucleic acids, respectively. The amendment is supported on paragraph [0035] of the specification. Accordingly, no new matter is introduced by the amendments and their entry is respectfully requested.

Claims 5-8 and 14 have been cancelled without prejudice. Applicants reserve the right to pursue the original subject matter of the claims in a continuation application.

The Examiner rejected claims 5, 7 and 8 under 35 U.S.C. 103(a) as allegedly being unpatentable over Becker in view of Amexis.

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

Applicants have amended claim 1 as described, *supra*. Applicants also submit herewith a Declaration by Dr. Chunming Ding ("Declaration"), a skilled artisan, setting forth reasons why a skilled artisan would not have considered the present method by reading Becker in view of Amexis.

The present claims are directed to absolute quantification of more than one nucleic acid in one reaction. Step a) of claim 1 reads:

> a) preparing a sample by combining in a sample the biological sample comprising the **at least two target nucleic acid sequences** and a known amount of at least two standard nucleic acids, wherein said at least two standard nucleic acids have a nucleotide sequence that is one base different than the respective target nucleic acid sequence;

Both Becker and Amexis are looking at a **single target** in a **uniplex reaction**. [See also Par. 6 of Declaration].

Becker quantified a nucleic acid using a standard that differed by one nucleotide so that a restriction enzyme would digest one of the amplified nucleic acids. [Par. 9 of Declaration].

Becker analyzed the amount of only one target nucleic acid, i.e., one nucleic acid molecule, per reaction. [Par. 10 of Declaration].

Amexis tested two allelic virus variants of the **same nucleic acid sequence** that differed from each other in one reaction. See, e.g., page 12100, first col., last paragraph. Amexis did **not** use a **standard** and thus the measurement was a relative quantification of the amount of one variant against the other. [Par. 7 of Declaration]. Thus in the Amexis reaction only one set of nucleic acids for a **single** target is used, not two targets and two standards. The present claims look at target sequences and their respective standards

As described in Table 1, lower part, Amexis used only one extension primer in each reaction. Therefore, the maximum number of extension products in each reaction was two if both alleles were present. The authors did not combine the analysis of multiple or even two different polymorphic markers in the same reaction. [Par. 8 of Declaration].

Amexis' discussion of overcoming problems in certain quantification reactions using enzymatic reactions by the use of MassArray technology with a primer extension reaction (see, e.g., page 12101, second column, last paragraph), does not in any way teach or suggest that the method can be used to look at multiple targets in a sample in a **single** reaction, i.e., using a multiplex method. As described above, Becker does not teach multiplexing either. [Par. 11 of Declaration].

As explained by Dr. Ding, both articles cited by the Examiner simply describe a uniplex system of nucleic acid quantification. [Par. 12 of Declaration].

The MassArray detection system is a very sensitive detection method. Therefore, this system was thought to be very sensitive also to artifacts in detection mixtures. [Par. 13 of Declaration].

In the present application, the Applicants tested, and surprisingly found that they were able to look at and accurately quantify, a plurality of sets of target nucleic acid sequences, i.e., more than two targets. The Applicants also found that we can accurately quantify multiple target sequences in the same reaction. The Applicants have used gene expression quantification for multiple targets. The declaration shows an example with a triplex PCR of three groups of sequences IL6, mcl1 and glut3 that were all co-amplified with their respective standards in the same reaction for PCR and primer extension (i.e. six sequences). Thus, the reaction mixture contained six different nucleic acids (3 sets of sequences with their respective standards). The Applicants found, that the extension products were clearly separated in the mass spectrum and

quantified by their peak areas. It was evident from the mass spectrum that high level of multiplexing can be achieved because our triplexing led to no deterioration in the precision of the method. This permitted one to further increase the throughput and reduce cost for large scale gene expression analysis. [Par. 14 of Declaration].

Exhibit A of Declaration shows one example of multiplexing real competitive PCR (rcPCR) assays which was performed prior to the filing of the current application. Genes IL6, mcl1 and glut3 and their respective standards were co-amplified in the same reaction and the mass spectrum is shown here. "P" following the gene name stands for not extended extension primer. "S" stands for the extended oligo from the standard DNA. C, G and A stand for the specific alleles of the three genes containing a C, G, or A base at the mutation site. The quantification results by multiplex reactions agreed well with those from uniplex reactions. [Par. 15 of Declaration]. Applicants report that the method has been used to quantify at least about 20 targets in one multiplex reaction. [Par. 16 of Declaration].

In paragraph [008] of the specification, Applicants teach that a plurality of target nucleic acids can be determined in a biological specimen. See also [0035]. [Par. 17 of Declaration]

Accordingly, contrary to the Examiner's argument, multiplex quantification, particularly using an extremely sensitive detection method such as mass spectrometric analysis, was not something scientists did or considered prior to the discovery by the Applicants. Typically, one would have expected to run into severe problems because of background noise if multiple peaks were to be analyzed in the same reaction. [Par. 18 of Declaration].

Therefore, Applicants submit that Becker does not teach or suggest quantification of at least two target nucleic acids with two standard nucleic acids in one sample. Amexis does not overcome this deficiency because Amexis similarly does not teach or suggest analysis of more that two target nucleic acids using a standard for each of them in one reaction.

In light of the amendments and the arguments presented above, Applicants respectfully submit that the rejection under 35 U.S.C. 103(a) over Becker in view of Amexis should be wishdrawn.

In view of the foregoing, Applicants respectfully submit that all claims are in condition for allowance.

Early and favorable action is respectfully requested. Examiner is encouraged to contact the undersigned attorney should there be additional questions regarding the application.

In the event that any additional fees are required, the Commissioner is hereby authorized to charge Nixon Peabody LLP deposit account No. 50-0850.

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Respectfully submitted,

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